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EMBRYOLOGICAL DEVELOPMENT OF THE SYLLID, AUTOLYTUS FASCIATUS (BOSC) (CLASS POLYCHAETA) [1]

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The Syllidae are unusual among the polychaetes in that some genera of this family reproduce both asexually, by the formation of stolons from a parent stock, and sexually, by the union of gametes from male and female individuals which, in turn, originate from stolons produced asexually. In some instances in this family, some form of gestation accompanies sexual reproduction—the young may develop in a ventral brood sac formed by the female, or eggs and larvae may remain individually attached to the parent (external gestation). A number of papers have been written on reproduction in syllids, and Saint-Joseph (1887) summarized the reproductive methods in the genus *Autolytus* and in the family as a whole. Since then, Potts (1911) has reviewed in considerable detail the various reproductive methods used by the four subfamilies of syllids, and recently Pettibone (1963) has given a brief summary of their reproductive methods. However, surprisingly little has been published on the embryology of this very interesting family of polychaetes, and most of this was written 50 to 120 years ago. Cleavage has been described and pictured in only a few papers on syllid reproduction (for example, Malaquin, 1893, the most extensive paper on syllids; Viguier, 1884—only the first three cleavages are shown; Pierantoni, 1903; and Schneider, 1914). Descriptions and figures of syllid larvae are scattered throughout the literature (Oersted, 1845; Müller, 1855; Agassiz, 1863; Pagenstecher, 1863; Greeff, 1879; Viguier, 1884; Saint-Joseph, 1887; Pierantoni, 1903; Herpin, 1926; Okada, 1930; Dales, 1951; *et al.*). However, descriptions depicting a series of developmental stages from early cleavage through several larval stages for any one species of syllid are rare. The writer has noted only two such descriptions in the literature (Viguier, 1884, and Pierantoni, 1903) and these belong to a different subfamily (Exogoninae) than does *Autolytus* (Subfamily Autolytinae). All figures in these two papers appear to be drawings of external views, and in Viguier's paper, the series on *Exogone gemmifera* does not include the stages between 8-cells and gastrulation.

[1] This research was supported by grants G-8775 and G-19958 from the National Science Foundation.

The writer has found embryological stages of *Autolytus fasciatus* abundant in Puerto Rican waters (Allen, 1957a, as *A. ornatus;* see Pettibone, 1963, for synonymy) and has reported observations on their histochemistry (Allen, 1957b, 1961). Since there appears to be no description of the embryological development of *A. fasciatus* in the literature, it seems advisable to publish the material now available on the normal embryology of this species, both to serve as background for continued histochemical studies and as an addition to our rather sparse knowledge of the development of this interesting group of polychaetes.

The genus *Autolytus* shows a striking sexual dimorphism (first clearly demonstrated by Agassiz, 1863, for *A. cornutus*) in the individuals that produce the gametes, the female often being referred to as the sacconereis form and the male as the polybostrichus. According to Potts (1911), gestation occurs in all forms of the subfamily Autolytinae. In the case of *Autolytus fasciatus*, the female, or sacconereis, forms a ventral sac which encloses the embryological stages from the fertilized egg through various differentiating larval stages (Fig. 25). This sac is often referred to as an egg sac although the term "brood sac" is perhaps a more accurate and more descriptive term. The present paper deals with the development of embryological stages within the ventral brood sac of the red-banded *Autolytus, A. fasciatus.*

Materals and Methods

Procuring and handling living developmental stages. As noted earlier (Allen, 1957a), night collections of the sacconereis or female can be made at almost any time of the year at the laboratory dock on the island of Magüeyes, located off La Parguera, Puerto Rico. Collections were made by use of a reflector bulb and a dip net. Individuals rise to the surface within the circle of light from a depth of several feet. When abundant, as many as 50 females (measuring approximately 13 mm. in length) have been collected with one dip of the net, and as many as 165 have been collected within an hour. During the year 1955, the females with ventral brood sacs were found to be most abundant during the last half of May and June, the middle of July, and the first part of September (Allen, 1957a); again in May and in early September of 1963 they were found to be abundant.

The brood sac of this species often gives the impression of having two, or more often three, lobes in linear arrangement. The ventral sac of any one female was found to contain just one developmental stage (Fig. 25); individuals could be seen through the thin wall of the brood sac. Some indication of the stage of development can be obtained from the color of the stage (as seen *en masse*) within the brood sac. There are some variations but if the brood sac appeared white and small, it generally contained early to mid-cleavage stages, while if it appeared blue or deep lavender, it usually contained rather smooth-surfaced spherical stages (late cleavage to gastrulae, or sometimes elongating trochophores). The brood sacs tend to appear lighter again as the trochophores develop and elongate, and then become a creamy-yellow color as swimming larvae develop. During these later larval stages, observations made through a dissecting microscope reveal red dots which are the eyes of the developing larvae, and they, together with the white color of the larval bodies, give an overall yellowish color to the brood sac.

For photomicrographs and for a more detailed study of the living organisms,

developing stages were freed from the brood sacs by using a #5 watchmaker's forceps and a dissecting microscope. Isolated individuals could then be viewed under low power of a compound microscope. Some of the earlier stages were isolated in this way in small stender dishes and were allowed to develop for a period in order to obtain an estimate of the time it took to develop from one stage to another. For such studies, and for photomicrographs, the ciliated stages were slowed down with a little dry MS-222 (tricain) added with a dissecting needle to a drop of filtered sea water containing the larvae (optimal concentrations for quieting various larval stages were not determined).

Handling of fixed material. Isolated stages were sometimes fixed for sectioning but, in most cases, once the stage had been determined by slitting the brood sac, the whole female with her brood sac of developing stages was fixed.

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FIGURE 1. A camera lucida drawing of a prophase from an early trochophore (squash preparation, alcoholic HCl-carmine). Note the six pairs of chromosomes of the diploid set.

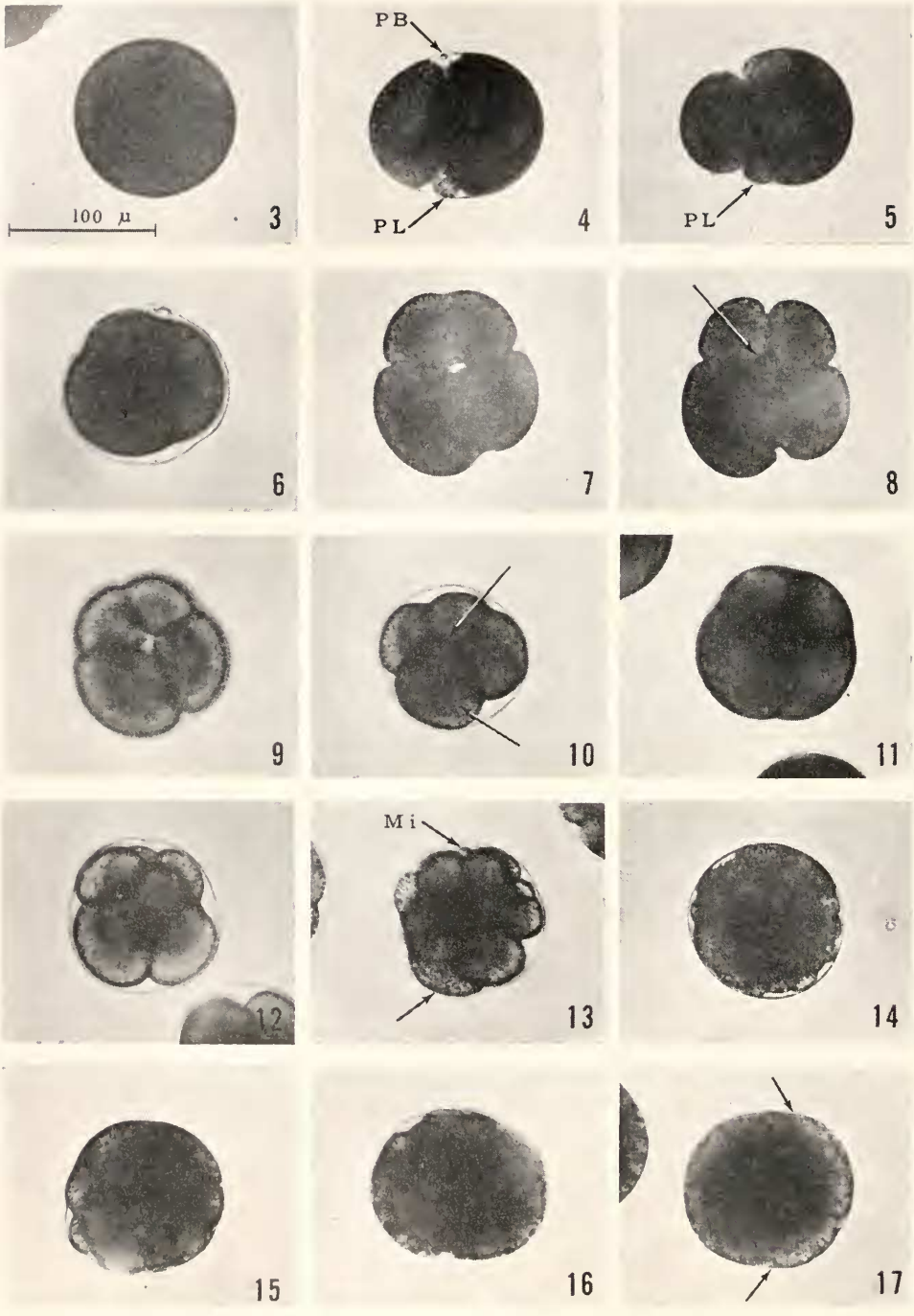
FIGURE 2. A camera lucida drawing of a polar view of a metaphase from an early trochophore (squash preparation, alcoholic HCl-carmine). Note again the 12 chromosomes of the diploid set.

The main fixatives used were acetic-alcohol (1:3), 80% ethyl or isopropyl alcohol, formal-calcium, picro-alcohol-formalin (Rossman's fixative), and Schaudinn's fixative. Most of the stages (usually while still in the brood sac attached to the female) were embedded in paraffin and sectioned serially at 5 to 8 micra; some, however, were stained as whole mounts. A number of stains were used including Harris' hematoxylin with or without eosin, azure B, gallocyanin chromalum, Pollak's trichrome, and fast green with Feulgen. Pollak's trichrome was particularly useful in determining the extent of the ciliated bands.

There seems to be no record in the literature for the chromosome number of any species of *Autolytus*, so in order to determine the diploid chromosome number for *A. fasciatus*, squash preparations of cleavage to early larval stages were made. Attempts at making preparations by the ordinary aceto-carmine technique for chromosomes were unsuccessful, but immediate success was obtained with the alcoholic HCl-carmine technique of Snow (1963). Photomicrographs and camera lucida drawings were made of the chromosomes.

DESCRIPTION OF DEVELOPMENT

Time table of development. It is difficult to construct a time table of development for *A. fasciatus* because the stages normally develop within the ventral brood



FIGURES 3–17.

sac attached to the female and the time of fertilization is unknown. However, an approximation was reached by two methods: (1) by liberating a few developing organisms from the sac and noting the stage of development, and then noting the time it took them to reach a later stage; (2) by carefully liberating a few embryos from the brood sac of a single female at various intervals and noting the progress of development. In the rare cases when undivided eggs were observed, it took them 30 minutes to two hours to cleave. Using this as a basis, the times given are approximations at temperatures of 25.5–29° C.

Stage	Time
Two-cells	2 hours
Four-cells	3½–4½ hours
Eight-cells	5–6 hours
Mid-cleavage	7–9 hours
Late cleavage	10–14 hours
Pre-trochophores	24–28 hours
Trochophores	1½–2 days
Post-trochophores	2½–3 days
Unhatched swimming larvae and hatching larvae	3½–6½ days

Chromosome number. Favorable views of squash preparations demonstrate that the diploid chromosome number for *A. fasciatus* is 12. The 12 chromosomes

Figures 3 through 23, except for Figure 20, are photomicrographs of living stages, all taken at the same magnification. The scale indicating magnification is shown on Figure 3 and is repeated on Figure 18. Figure 20 is a photomicrograph taken from a squash preparation. Figures 24 through 45 are photomicrographs of sectioned material. Figure 24 and Figures 28 through 43, except for Figures 36, 40, and 42, were all taken at the same magnification; the scale is indicated on Figure 28, and is repeated on Figures 30 and 38.

FIGURE 3. Unfertilized egg, with numerous yolk spheres making it so opaque that the germinal vesicle is not visible.

FIGURE 4. Two-cell stage, showing that the AB blastomere is somewhat smaller than the CD. Note the polar lobe (PL) and one of the polar bodies (PB).

FIGURE 5. Two-cell stage, showing the polar lobe (PL) being withdrawn into the CD blastomere.

FIGURE 6. Second cleavage furrow beginning. Note the two polar bodies held within the fertilization membrane.

FIGURE 7. Central hole appearing as the second cleavage furrow becomes evident.

FIGURE 8. Polar view showing a tongue-like extension of the D-blastomere. This extension (arrow) may represent a second polar lobe.

FIGURE 9. Four-cell stage showing distinct cleavage furrows and persistence of central hole.

FIGURE 10. Four-cell stage completed with the obliteration of the hole as the blastomeres move over one another to form the cross-furrow (at tip of upper arrow). Note that the D-blastomere (at lower arrow) is larger than the others.

FIGURE 11. First and second cleavage furrows becoming indistinct just prior to initiation of the third cleavage furrow.

FIGURE 12. Lateral view of eight-cell stage, showing two tiers with four slightly smaller micromeres towards the animal pole.

FIGURE 13. Early to mid-cleavage showing one of the smaller micromeres (Mi) and the largest macromere (lower arrow), probably the D-blastomere.

FIGURES 14 and 15. Late cleavage stages, showing closely packed cells, and outer edge becoming smoother as surface blastomeres become smaller.

FIGURE 16. Probably a normal gastrula stage although all batches of eggs do not show this irregular shape.

FIGURE 17. Very early trochophore (anterior at right). Arrows indicate where cilia of prototroch will penetrate the larval membrane.

may be counted in the prophase stage shown in Figures 1 (a camera lucida drawing) and 20 (a photomicrograph of the same prophase). A polar view of the metaphase in which all the chromosomes may be counted is shown in Figure 2 (a camera lucida drawing). These three figures were made from squash preparations of early trochophores. So far as the writer has determined from the literature, the chromosome number of no other species of *Autolytus* has been determined.

Unfertilized eggs. Before being released into the brood sac, white spherical eggs, measuring approximately 100 micra in diameter, are packed within the coelom of the female along the entire length of the body. Hartman (1945) describes the eggs of this species as bright blue but she must have been referring to later spherical stages as described above; this undoubtedly also accounts for Pettibone's reference (1963, page 142) to these eggs as "whitish, bright blue or purplish." No polar bodies were observed in these coelomic eggs, and sectioned material reveals that most of the oöcytes are in metaphase I (Fig. 28). Presumably the oöcytes remain in this phase until fertilization occurs, for polar bodies have not been noted until after fertilization when they were observed in sectioned material of fertilized eggs within the brood sac (Figs. 24 and 29; note spermatozoan heads in Figs. 24 and 27). A photomicrograph of a living egg is shown in Figure 3. Viewed with the light microscope, the cytoplasm of the egg in sectioned material appears as a network of fine granules surrounding the closely packed yolk spheres (compare Figs. 26, 28, and 30—yolk spheres are stained only in Fig. 30). The cytoplasmic granules are more concentrated peripherally, forming a thin cortical layer (compare Figs. 26 and 28). This cortical layer is bounded by a thin transparent vitelline membrane which is difficult to see except where it has been pulled away from the egg surface (Fig. 26).

Fertilization. Fertilization was not observed in the present investigation, the male form having been seen only rarely, and usually not at the dock where females were collected but over a nearby reef. Spermatozoa, however, were observed in sectioned material within egg sacs containing undivided eggs or early cleavage stages. These spermatozoa may be seen associated with the eggs (Fig. 24) or stuck to the inside of the egg sac (Fig. 27—the two sac-like protrusions on the midpiece of the lower spermatozoön may be an abnormality).

The writer has found only a few references to fertilization in syllids and no exact description of the process. Fertilization in most instances appears to be external, occurring either after swarming and release of gametes into the sea or, in cases of external gestation, after the eggs have been released and attached externally to the female (Viguier, 1884; Pierantoni, 1903; Galloway and Welch, 1911; Herpin, 1923, 1924a, 1924b, 1926; *et al.*).

In the Subfamily Autolytinae, Herpin (1926) describes the ventral brood sac as being formed by the female at the moment of egg laying. L. Dehorne (1918, from Gravier, 1923) described and pictured the formation of the egg sac in *Myrianida;* as the egg sac is secreted, the eggs pass through the modified nephridia of the female into this ventral sac. They thus must become fertilized before the egg sac is completed. According to Gidholm (personal communication; and 1964, Zool. Bidr. Uppsala, in press), fertilization in *Autolytus edwarsi* is external; the sexual forms swarm at the surface and the male swims rapidly around the female ejecting "a mucous sperm which forms a belt around the female." The

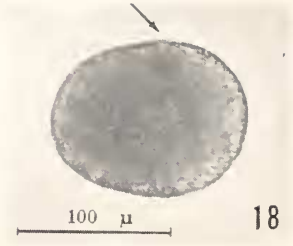
eggs are not exuded immediately and the brood sac is formed by mucous glands in the bases of the parapodia; as the eggs exude from genital ducts (modified nephridial ducts), they are fertilized immediately. The eggs are shed in metaphase I and upon sperm entrance form two polar bodies, thus completing the meiotic divisions. Gidholm has observed mating behavior in the laboratory in several other species of *Autolytus* besides *A. edwarsi*, so he feels that the above description is probably typical of the genus. It seems likely, then, that this description of fertilization would apply to *A. fasciatus*.

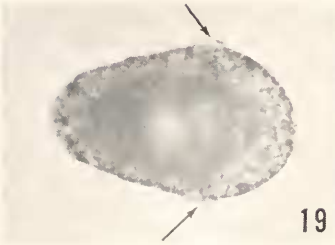
As in a number of other annelids, no raised fertilization membrane is produced in *A. fasciatus* (Figs. 4, 7, and 8). The polar bodies as a consequence appear to lie for a time in a depression on the egg surface (Fig. 29). Sections of a small clump of uncleaved eggs revealed the male and female pronuclei prior to the formation of the first cleavage spindle (Fig. 30).

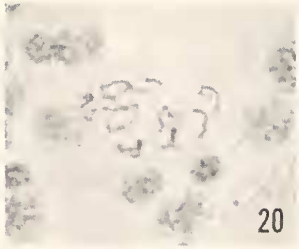
Cleavage. Females with uncleaved eggs in the brood sac, or with two-cell stages, were extremely rare in the collections made. However, in the spring of 1963, several such females were observed and were used to obtain further information regarding early cleavage. A series of photomicrographs was made as these living eggs cleaved. Cleavage is total, unequal, and spiral. The blastomeres are held closely together by the vitelline membrane (obvious only at the cleavage furrows) so that they are somewhat flattened against one another. This flattening of the blastomeres is not very marked in some of the photomicrographs of living stages (Plate I); in these stages it is probable that the heat of the lamp caused the egg membrane to swell slightly so that the blastomeres rounded up more than is typical. This, however, had the advantage of making the early cleavage pattern and spatial relationships of the blastomeres easier to determine. Eggs and blastomeres are so opaque that chromosomes and spindles were not observed in living material.

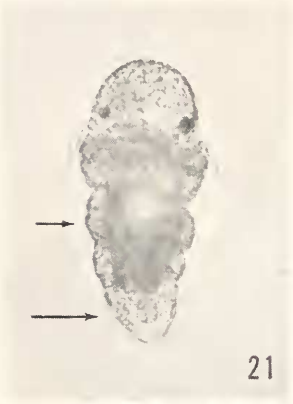
The first cleavage furrow is meridional, resulting in two blastomeres of unequal size, the AB being somewhat smaller (approximately 90×50 micra) than the CD blastomere (approximately 100×70 micra, Fig. 4). At this two-cell stage a polar lobe may be observed at the vegetal pole; it is subsequently gradually withdrawn into the CD blastomere (compare Figs. 4 and 5). As the second cleavage furrow forms, a hole appears between the cleaving blastomeres (Fig. 7), and then a tongue-like extension of the D-blastomere was sometimes observed in the region where the cross-furrow will form (Fig. 8). Although material was scarce, the few cases observed suggest that the tongue-like extension may represent the formation of another polar lobe as seen from a vegetal pole view (compare Fig. 8 with Wilson, 1904, Fig. III, 15 for *Dentalium*).

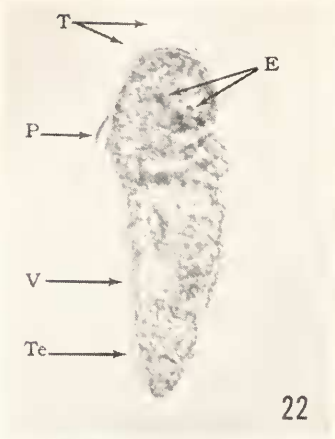
During the first and second cleavages in some molluscs (for example, *Ilyanassa* and *Dentalium*) strikingly large polar lobes are formed (Raven, 1958, Fig. 19 after Morgan; and Wilson, 1904, Figs. I and II). The formation of polar lobes seems to be relatively rare in polychaetes. In the case of *Autolytus* described here, the polar lobes are more nearly the size of the relatively small ones formed in the early cleavage of the polychaete, *Chaetopterus* (compare Figs. 4 and 5 with Mead, 1897, Plate XIX, Figs. 118 and 119). The only reference noted in the literature describing the formation of polar lobes in the cleavage of syllids was that of Schneider (1914) in which he diagrammed the first polar lobe (Fig.

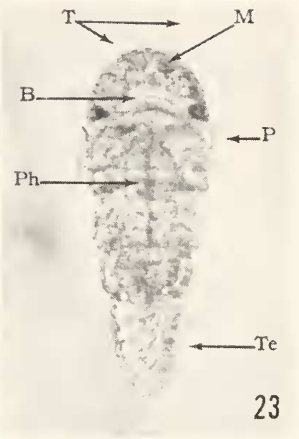


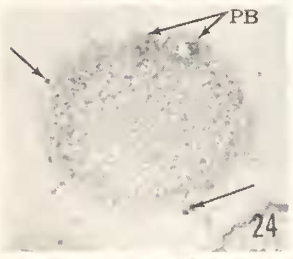


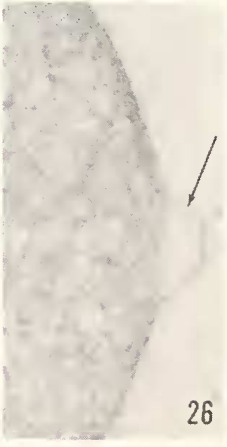


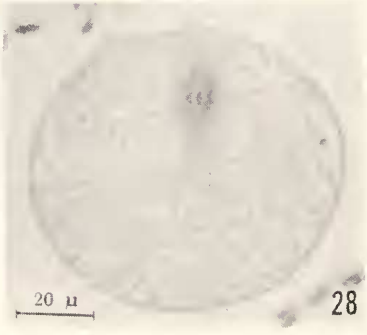


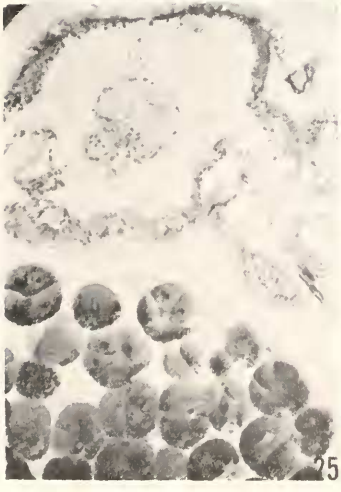


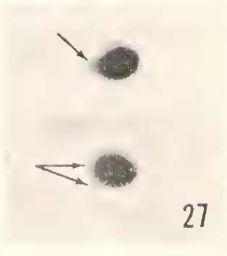


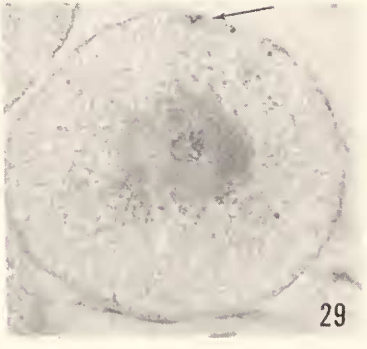












FIGURES 18–29.

4, page 625) and referred to the formation of a second (page 625). This short paper by Schneider is concerned with the early development of *Pionsyllis pulligera* which belongs to the subfamily Eusyllinae, a different subfamily from that to which *Autolytus* belongs (Subfamily Autolytinae).

The hole which may be observed as the second cleavage furrow forms (Fig. 7) remains until the furrows of the four-cell stage become distinct (Fig. 9) but is subsequently obliterated as the blastomeres move over one another to form the cross-furrow (Figs. 10 and 31) which is typical of spiral cleavage. The resulting C and D blastomeres are larger than the A and B cells, the D blastomere being the largest (Fig. 10).

Foreshadowing the initiation of the third cleavage furrow, the two furrows of the four-cell stage become indistinct (Fig. 11). The third cleavage furrow is horizontal; its completion results in a tier of four somewhat smaller micromeres lying over four macromeres (Fig. 12; compare with Malaquin, 1893, Plate XIV, Figs. 4 to 10, in which the size discrepancy between early micromeres and macromeres is much greater in the closely related genus, *Myrianida,* than in *Autolytus*). The large blastomere which persists as cleavage continues is probably the D macromere (Fig. 13) and presumably gives rise to the mesoderm of the larva. Later cleavages are asynchronous (Figs. 13 to 15, and 32); the

FIGURE 18. Dorsal view of an early trochophore without eyes (anterior to right). Arrow indicates beating cilia of the prototroch which have penetrated the larval membrane.

FIGURE 19. Dorsal view of an older trochophore showing prototroch (arrows), two eyespots just anterior to the prototroch, and darker central yolk mass with a pharynx differentiating at the level of the prototroch.

FIGURE 20. Squash preparation from an early trochophore stage showing a prophase with the diploid set of twelve chromosomes. Alcoholic HCl-carmine.

FIGURE 21. Dorsal view of an elongated post-trochophore with two prominent eyespots, three ciliated areas (prototroch is clear; arrows indicate level of more posterior cilia), and differentiating central gut. Anterior tactile bristles are present but are not clearly shown.

FIGURE 22. Lateral view of late unhatched swimming larva, showing tactile bristles (T), two of the four eyes (E), prototroch (P), telotroch (Te), and beating ventral cilia shown as hazy line (V).

FIGURE 23. Dorsal view of a partially contracted larva similar to that in Figure 22, showing anterior tactile bristles (T), anterior mucous cells (M), brain (B) between the two larger eyes, prototroch (P), pharynx (Ph), and telotroch (Te). The middle cilia are not visible.

FIGURE 24. Section through a fertilized egg near the animal pole, showing the two polar bodies (PB), and two sperm heads (visible at arrows—the larger dark spot at lower arrow is not a sperm). Gallocyanin.

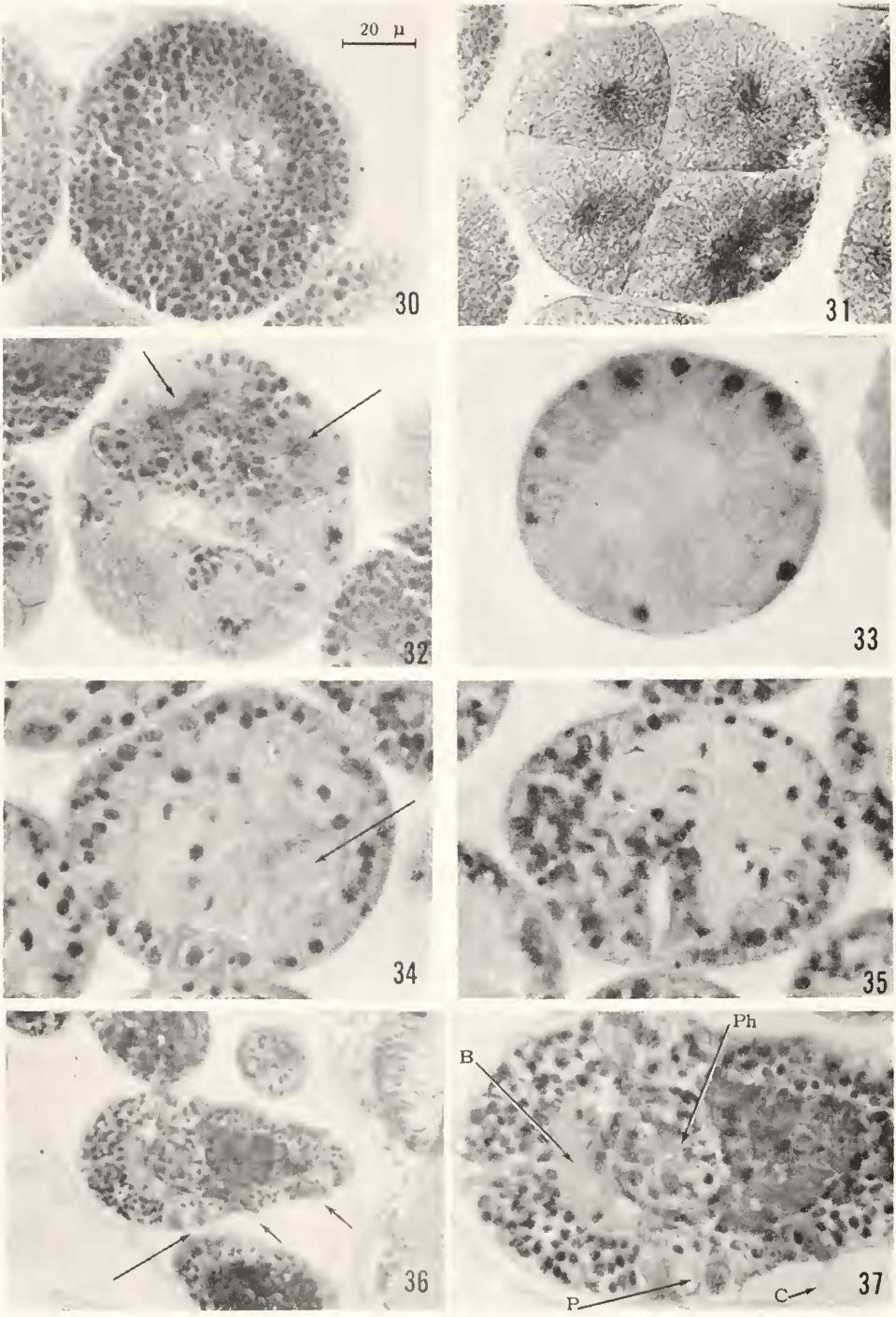
FIGURE 25. Transverse section through a female, or sacconereis, showing a portion of the ventral brood sac packed with early cleavage stages. Pollak's trichrome.

FIGURE 26. Enlargement of a portion of a sectioned egg, showing a network of fine granules surrounding the closely packed yolk spheres (yolk is unstained) and a cortical concentration of granules. Note the thin vitelline membrane where it has been pulled away from the surface (at arrow). Gallocyanin.

FIGURE 27. Two spermatozoa caught on the inner mucous surface of the brood sac and seen in sectioned material. The flagellum of the upper sperm was barely visible (arrow indicates lighter midpiece). The two sac-like protrusions on the midpiece (arrows) of the lower sperm may be an abnormality. Pollak's trichrome.

FIGURE 28. Primary oöcyte in metaphase I shown inside the coelom of the sacconereis (yolk is unstained). Gallocyanin.

FIGURE 29. A fertilized egg showing chromosomes in first cleavage. Note the two polar bodies (at arrow) lying in a depression on the egg surface, and the absence of a raised fertilization membrane. Gallocyanin.



FIGURES 30–37.

opaqueness of the blastomeres, due to their stored yolk, makes it very difficult to work out the details. The apical rosette and annelid cross, characteristic of polychaete development, have occasionally been observed in living material. Late cleavage to blastula stages appear in living material as spheres of closely packed small cells with the outer edge becoming smoother as surface blastomeres become smaller (compare Figs. 14 and 15).

Gastrulation. Both living stages and sectioned material indicate that gastrulation is accomplished by epiboly, the micromeres growing over the endodermal macromeres (compare Figs. 33 to 35), much as in the case of *Sphaerosyllis* (Pierantoni, 1903, Plate II, Figs. 19 and 20) and *Myrianida* (Malaquin, 1893, Plate XIV, Figs. 7 to 10). The gastrula of *Autolytus fasciatus* may appear in the living state as somewhat irregular in shape (Fig. 16). The subsequent larval stages are described below.

Trochophores. Young trochophores are ovoid but are becoming broader anteriorly where the prototroch is differentiating (compare Figs. 17 and 18). In living stages the prototroch is first observed as a thickened area around the broadest part of the anterior end. In the larva shown in Figure 17, the cilia of the prototroch have not yet penetrated the larval membrane. As individual cilia penetrate the membrane (Fig. 18), they begin to beat, and soon the trochophore is moving about in place. A telotroch also is beginning to differentiate but is so finely ciliated at this stage that it is difficult to observe in younger members of this stage, such as the larva in Figure 18. *A. fasciatus* appears to be precocious in the development of these ciliated bands, compared with *A. edwarsi* (Malaquin, 1893, Plate XIV). The younger stages have not yet developed eyes and the older ones have two orange-red eyespots just anterior to the prototroch (compare Figs. 18 and 19). Centrally, just behind the prototroch, the larval pharynx is differentiating; in sectioned material, the stomodeal invagination is clearly seen (Fig. 35). The post-pharyngeal gut is obvious in living and sectioned material as a dense central mass of endodermal cells, tapering posteriorly and packed with yolk spheres (Figs. 19 and 35). The cilia of this and later stages appear to penetrate the original egg membrane which has thus become the larval cuticle; as noted earlier (Allen, 1959, *et al.*) this is not un-

FIGURE 30. Fertilized egg, showing male and female pronuclei near center. Yolk spheres are stained. Pollak's trichrome.

FIGURE 31. Four-cell stage, showing cross-furrow in center. Pollak's trichrome.

FIGURE 32. Early cleavage stage. Note anaphase at upper left (arrow) and polar view of metaphase at upper right (arrow). Pollak's trichrome.

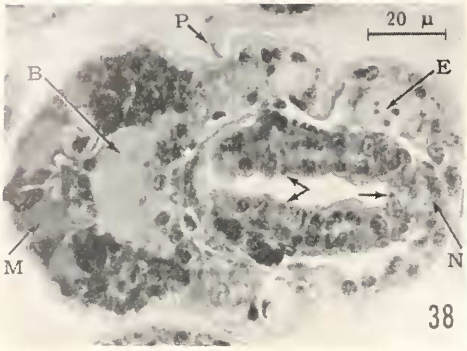
FIGURE 33. Later cleavage stage. Note epibolizing micromeres, and central yolk mass. Harris' hematoxylin and eosin.

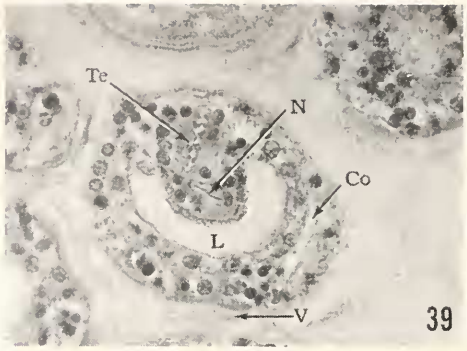
FIGURE 34. Stage just prior to stomodeal invagination. Note anaphase in an endodermal cell (arrow). Harris' hematoxylin and eosin.

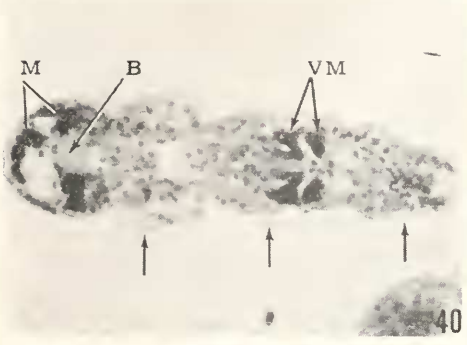
FIGURE 35. Sagittal section through an early trochophore without eyes (anterior at left), showing stomodeal invagination. Note central mass of yolky endodermal cells. Harris' hematoxylin and eosin.

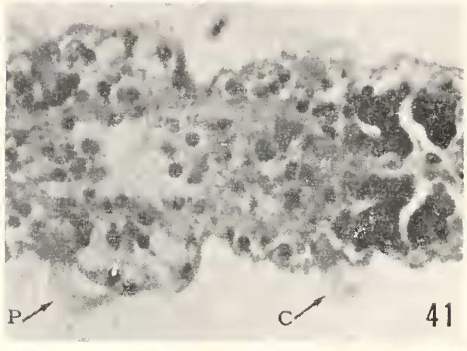
FIGURE 36. Frontal section through a post-trochophore (anterior at left), showing three ciliated areas (arrows), brain, pharynx, and undifferentiated post-pharyngeal gut showing as a dark central yolk mass. Section does not include mucous cells. Feulgen and fast green.

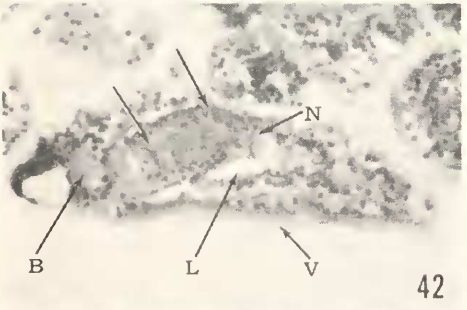
FIGURE 37. Enlargement of anterior two-thirds of post-trochophore shown in Figure 36. Note differentiating brain (B), pharynx (Ph), large prototrochal cells (P), and lateral ciliated tuft (C).

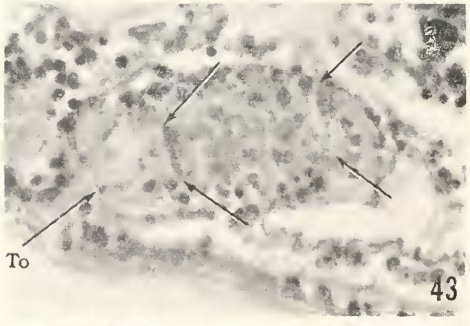


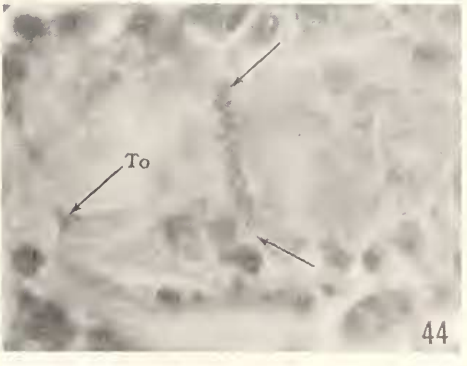


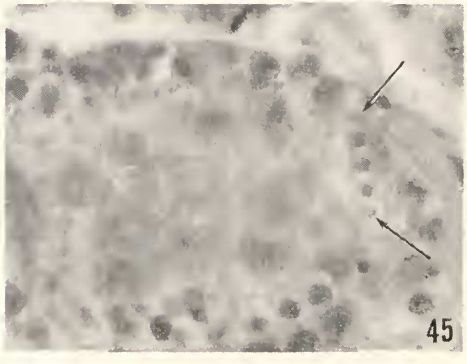












FIGURES 38–45.

common among polychaetes. Several investigators (Barrois, 1877; Viguier, 1884; Malaquin, 1893; Thorson, 1946) have noted this origin of the larval cuticle in connection with the development of syllids. Pierantoni (1903), however, does not agree with the interpretation that the larval cuticle represents the original egg membrane.

Post-trochophores. This stage has elongated, and a pair of lateral ciliated tufts has developed between the active prototroch (a complete band of long cilia) and telotroch (Figs. 21, 36, and 37). The ventral surface has also developed cilia. Similar ventral cilia have been pictured for *A. cornutus* (Dales, 1951, as *A. prolifer*—see Pettibone, 1954) but not for *A. edwarsi* (Malaquin, 1893). The larvae move slowly about on the bottom and the more active are able to swim to the surface, rotating about their longitudinal axes. Younger members of this group still have only two eyespots, but the older larvae are developing a second pair of eyes, and also have at least two slender spine-like structures extending from the anterior surface (not clear in Fig. 21).

In addition to the above, sectioned material reveals that a number of large cells, probably mucous (Allen, 1961), have differentiated at the anterior tip of the head. The brain is differentiating, and the pharynx and post-pharyngeal gut are clearly defined although the latter is still relatively undifferentiated (Figs. 36 and 37).

Late unhatched swimming larvae and newly hatched larvae. The brood sacs containing these unhatched larvae are usually large and appear pale yellow in color. The late unhatched and newly hatched larvae are essentially similar, but the hatched ones are very active swimmers and are positively phototactic, collecting at the edge of the dish nearest the light, thus making it easy to remove them for fixation or for changing the sea water. These swimming larvae measure approximately 300 × 100 micra; the ventral surface is covered by cilia (Figs. 22, 39,

FIGURE 38. Frontal section through anterior region of a late unhatched swimming larva, showing large mucous cells (M) and brain (B) in the cephalic segment, and pharynx (with pharyngeal teeth indicated by central arrows). Note also the prototroch (P), the large endodermal sphere (E, containing yolk granules) within the wall of the intestine, and the narrow post-pharyngeal area of the gut (N) shown in transverse section.

FIGURE 39. Transverse section through a larva similar to that in Figure 38, showing the posterior end of the pharynx with two lateral plates of pharyngeal teeth (Te), the narrow area of the gut (N) pushing into the lumen (L) of the anterior part of the intestine. Note also the coelom (Co) between intestine and body wall, and the ventral cilia (V).

FIGURE 40. Frontal section through a larva similar to that in Figures 38 and 39, showing anterior mucous cells (M), brain (B), and group of prominent ventral mucous cells (VM). Note also the levels of prototroch, lateral ciliated tuft, and telotroch (arrows).

FIGURE 41. Enlargement of central portion of the larva shown in Figure 40, with prototroch (P), lateral ciliated tuft (C), and ventral mucous cells.

FIGURE 42. Sagittal section through a larva similar to those in Figures 38 to 41, showing the brain (B), and pharynx with anterior and posterior pharyngeal teeth (level of arrows). Note also the narrow section of the gut (N), and lumen (L) of anterior part of intestine. The hazy layer on the lower surface represents the ventral cilia (V).

FIGURE 43. Enlargement of pharynx shown in Figure 42. Note single large tooth (To) near the mouth, the double row of anterior teeth (between anterior pair of arrows), and row of five posterior teeth (between posterior arrows).

FIGURE 44. Enlargement of single anterior tooth (To), and double row of anterior teeth (between pair of arrows) shown in Figure 43.

FIGURE 45. Enlargement of the five posterior teeth (between arrows) shown in Figure 43.

42, and 43). The larvae have a segmented appearance but the segments are not yet completely demarcated. These early larval segments are described in the following paragraphs. Most of the structures described can be observed in living material.

(1) *Cephalic segment.* The cephalic segment bears two pairs of red eyes, one pair being smaller and located slightly posterior, median, and dorsal to the larger pair; each of the larger pair has developed a lens. The differentiating brain can be observed in living larvae as a whitish area between the two larger eyes (Fig. 23) and is clearly seen in sections (Figs. 38, 40, and 42). Anterior to the brain several large mucous cells are arranged symmetrically in a curved row following the external contour of the head (Figs. 23, 38, and 40). In favorable preparations of living larvae, both nucleus and nucleolus may be observed in these cells. Several anterior slender spine-like structures are visible on the head (Figs. 22 and 23). Similar structures are shown in the drawings of larvae of several other species of syllids (Greeff, 1879; Malaquin, 1893; Herpin, 1926; Okada, 1930; Dales, 1951). Some investigators refer to them as tactile hairs or bristles (Herpin, 1926, described them as "poils tactiles" or "cils tactiles raides"; Greeff, 1879, uses the term "Tastborsten" or "Hautstacheln"). In favorable preparations of living specimens, close examination of these tactile bristles under a magnification of × 430 revealed that they are actually very slender tufts of fine cilia. Thus, structurally they suggest a slender version of the cirri characteristic of certain protozoon ciliates (order Hypotricha). Two minute short anterior tufts of cilia may also be observed just median to these anterior tactile bristles, and also a minute median tactile bristle can be seen between these two small tufts. These latter cilia are so small and delicate they do not show up in the photographs. Short, fine cilia can be seen just anterior to the prototroch; they are difficult to observe clearly but appear to arise from the posterior edge of the cephalic segment.

(2) *Anterior pharyngeal segment* and (3) *Posterior pharyngeal segment.* The band of short cilia described in the preceding paragraph appears to be almost confluent with the prototroch, which is the prominent band of longer, very active cilia borne on the anterior part of the anterior pharyngeal segment (Figs. 23, 38, and 41). Just posterior to the prototroch is a pair of fragile-looking spine-like structures which are probably similar in structure to the anterior tactile bristles; similar lateral tactile bristles have been pictured in other syllid larvae of the subfamilies Autolytinae and Eusyllinae (Malaquin, 1893; Herpin, 1926; Okada, 1930).

Internally, at the level of the prototroch, the anterior edge of the pharynx is visible (Figs. 23 and 38). The pharynx extends through the rest of this segment and through the anterior part of the posterior pharyngeal segment (Figs 23 and 38), where it narrows to form a short section (Figs 38, 39, and 42). This short narrow portion, in turn, joins the differentiating intestine which has developed a relatively large lumen at this level (Figs. 39 and 42).

The spatial relationships of the larval pharynx, the anterior end of the intestine, and the narrow portion of the digestive tract in between, arise in the following way. During its formation, the stomodeal invagination pushes into the anterior gut endoderm. Subsequently, the larval pharynx differentiates from the

invaginated ectoderm, and the intestine differentiates from the yolk-filled endoderm. In the meantime, the narrow portion—if its origin is similar to that described for other larval syllids (Malaquin, 1893, *et al.*)—arises from a posterior proliferation of the larval pharynx. As a consequence of these processes, the anterior end of the intestine in these swimming larvae surrounds the posterior end of the larval pharynx (Figs. 38, 39, and 42), and the narrow post-pharyngeal gut appears to push into the anterior portion of the differentiating intestine (Figs. 38 and 39). This narrow section between the larval pharynx and the differentiating intestine may represent the primordium of the posterior part of the adult pharynx, together with the proventriculus and ventriculus, as the subsequent development of this area has been described for other syllids (Malaquin, 1893; Pierantoni, 1903; Herpin, 1926), including *Autolytus edwarsi* (Malaquin, 1893). It will be necessary, however, to study later larval stages before this can be confirmed for *Autolytus fasciatus*.

Large spheres seen at the level of the pharynx superficially suggest the precocious development of eggs. However, they are associated with the lining of the intestine (Fig. 38); position and staining reactions suggest them to be the remains of some of the yolk-filled endodermal cells of the post-trochophore stage rather than developing eggs.

The posterior pharyngeal segment bears a pair of prominent ciliated tufts (Figs. 40 and 41) described above as appearing in the post-trochophore stage. Just anterior to these lateral tufts of cilia is a second pair of tactile bristles (similarly placed bristles are shown for *A. pictus* in Okada, 1930, Fig. 2A). Internally, pharyngeal teeth are differentiating. At least two relatively large teeth may be seen at the anterior end of the pharynx (one of these is shown in Figs. 43 and 44). About one-third the length of the pharynx, a circle of teeth has differentiated (one lateral set of these teeth is shown in Figs. 43 and 44). Near the posterior end of the pharynx, there is a set of somewhat stouter teeth arranged in two lateral halves (compare Figs. 43 and 45 with 39). Six teeth have been counted in each half of this posterior set (five are visible in Fig. 45). The narrow post-pharyngeal gut is also differentiating teeth, but the details were difficult to observe.

(4) *Narrow segment with no ciliated band.* Externally, this segment has ventral cilia and a pair of lateral tactile bristles comparable to the two pairs described above. Internally, the differentiating intestine passes through this segment. The lumen of the anterior part of the intestine is relatively large and the coelom is becoming well-defined (shown more anteriorly in Fig. 39). The beating cilia occasionally observed internally in this area originate from the cells lining the gut.

(5) *The anal segment or pygidium.* The pygidium is a relatively long segment, narrowing to the posterior tip of the larva. It bears the telotroch (Figs. 22, 23, and 40), first described above in connection with the trochophore stage. In favorable preparations of living larvae, a small pair of tactile bristles can be observed at the posterior end (posterior bristles are also pictured by Okada, 1930, for several species of *Autolytus*, and by Malaquin, 1893, for *A. edwarsi*). In the midline between these tactile bristles, at the posterior tip of the larva, is a group of short cilia giving the impression of a minute flattened brush. At least

some of these cilia have their origin anterior to the tactile bristles. Internally, the intestine of the anal segment has not yet differentiated a lumen.

Hatched larvae. Larvae which had developed from several different batches of eggs were kept for several days after hatching, and observations were made on living stages. There was no evidence of setae or tentacular buds, as yet, and no other marked changes were observed.

Comparison of syllid larvae. Most of the descriptions of syllid larvae were written in the 1800s and the first three decades of this century. According to some of the workers of this period the descriptions and figures of others were incomplete and inaccurate. This makes a detailed comparison impossible. However, a few larval characteristics may be compared in a general way. In comparison with other species of the Genus *Autolytus*, *A. fasciatus* is further developed at the time of hatching. According to Agassiz (1863, Plate X, Fig. 2), *A. cornutus* is relatively undifferentiated both externally and internally at hatching, although Malaquin (1893) points out that the absence of cilia in Agassiz's description of this species is probably an error. According to Malaquin, *A. edwarsi* has two lateral pairs of ciliated tufts at hatching but does not develop the equivalent of a prototroch or form other ciliated bands until sometime after hatching, and he makes no mention of cilia covering the ventral surface. In *A. fasciatus* described here, three prominent ciliated areas, as well as cilia covering the ventral surface, are present for some time before hatching. The well developed cilia in this species enable the larva to swim actively and suggest that the larvae are pelagic, for a time at least, after hatching. It has been pointed out that members of other subfamilies of syllids may have a short pelagic existence (Thorson, 1946, *et al.*). Larvae of *A. edwarsi*, in which the formation of cilia is delayed, as compared with the relatively precocious development of cilia in *A. fasciatus*, appear to take up a creeping existence on bryozoans or hydroids almost as soon as they are hatched (Malaquin, 1893). Development of functional mucous cells in *A. fasciatus* suggests that the pelagic life of these larvae may be replaced shortly by a creeping existence with the ability to form slime tubes. The Exogoninae, on the other hand, appear to have no larval cilia and no pelagic existence (Viguier, 1884; Saint-Joseph, 1887; Malaquin, 1893; Pierantoni, 1903; Thorson, 1946).

Anterior tactile bristles similar to those described above for the larvae of *A. fasciatus* are shown in the drawings of several other species of *Autolytus* (Greeff, 1879; Okada, 1930; Dales, 1951) and can also be observed in certain members of the Subfamilies Syllinae and Eusyllinae (Herpin, 1926). They may appear relatively late, however (as in *Eusyllis*), which is also the case for external cilia (Malaquin, 1893). Three pairs of lateral tactile bristles have been described above for larvae of *A. fasciatus*. Okada (1930) showed paired lateral tactile bristles in several species of *Autolytus*, although Agassiz (1863) and Dales (1951) do not show them for *A. cornutus* (described as *A. prolifer* by Dales—see Pettibone, 1954). Paired lateral tactile bristles have also been demonstrated in certain Syllinae and Eusyllinae but not in others (Malaquin, 1893; Herpin, 1926). Apparently larvae thus far examined in the Subfamily Exogoninae lack these tactile bristles, as well as any marked external ciliation. Although tactile bristles are small and delicate so that their presence may have been missed by some inves-

tigators, they may prove to be a useful characteristic in the identification of syllid larvae.

As described above, the pharynx of hatching larvae of *A. fasciatus* has two anterior teeth and an anterior and a posterior circle of teeth. Investigations thus far indicate that larvae of the Subfamily Exogoninae lack pharyngeal teeth except for a single median anterior tooth (this is consistent with the structure of the adult pharynx—see Fauvel, 1923). Herpin (1926) described and pictured a circle of differentiated teeth (composed of dorsal and ventral halves) in the larval pharynx of *Odontosyllis ctenostoma* (Subfamily Eusyllinae). These may be comparable to the anterior circle of teeth described above for *A. fasciatus*. Malaquin (1893) does not mention a second circle of pharyngeal teeth in the larvae of *A. edwarsi;* however, a second circle of teeth is indicated in several of his drawings (see Plate XIV, Figs. 17a, 17b especially, and 18). Thus, the posterior circle of pharyngeal teeth may be a specific characteristic of certain members of the genus *Autolytus*. These posterior teeth may have been missed by several investigators, for Dales (1951) points out that larval pharyngeal teeth may be difficult to see. Should their specificity prove true, pharyngeal teeth may serve as a useful criterion for distinguishing between species of syllid larvae as they have between certain other polychaete larvae (Allen, 1959, *et al.*).

The larval stages of *A. fasciatus* which were studied had not yet developed setae and were still in the monopharyngeal stage. It will be necessary to study later stages before setal types and larval segmentation can be compared with other syllids.

The writer wishes to acknowledge the assistance of Mrs. Darthea B. Keith in making the histological preparations of serial sections.

Summary

1. A series of developmental stages, from undivided eggs through swimming larvae, were isolated from the brood pouches of the polychaetous worm, *Autolytus fasciatus* (the red-banded *Autolytus*), collected in Puerto Rican waters. Observations on both living stages and on fixed and stained preparations have been described and photographed.

2. The diploid chromosome number for this species was determined as 12. Fertilization occurs at metaphase I. The cleavage pattern is spiral and involves polar lobe formation. Gastrulation is by epiboly. The trochophore and later larval stages show a precocious development of active cilia as compared with other syllid larvae. Characteristic differentiations of larvae at hatching are anterior and lateral tactile bristles, anterior and ventral mucus-secreting cells, and an anterior pair and two circles of pharyngeal teeth. Larvae were compared with other syllid larvae as regards ciliation, tactile bristles, and pharyngeal teeth.

3. Insofar as the writer knows, the present study is the only one recorded in the literature on the early developmental stages of *Autolytus fasciatus*, and the only one on the Subfamily Autolytinae which includes a series of developmental stages from uncleaved eggs through various larval stages. Much more embryological work needs to be done on the Family Syllidae.

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